Waldenström Macroglobulinemia

Development of Diagnostic Criteria and Identification of Prognostic Factors

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Abstract

To establish whether a combination of morphologic and immunophenotypic criteria could be developed to more precisely define Waldenström macroglobulinemia (WM) and prognostic factors, we retrospectively assessed the clinical and laboratory features of 111 cases of WM. Bone marrow infiltration by small lymphocytes was documented in each case; and diffuse, interstitial, nodular, and paratrabecular patterns of infiltration were documented in 58%, 32%, 6%, and 4% of cases, respectively. Ninety percent were characterized by a surface immunoglobulin–positive, CD19+CD20+CD5–CD10–CD23– immunophenotype.

The median overall survival from diagnosis was 60 months; univariate analysis revealed the following adverse prognostic factors: older than 60 years, performance status more than 1, platelet count less than 100 × 10^3/μL (<100 × 10^9/L), pancytopenia, and diffuse bone marrow infiltration. Associated median survival was 40, 38, 46, 28, and 59 months, respectively. Multivariate analysis revealed age, performance status, and platelet count as prognostically significant, but stratification of patients according to the International Prognostic Index had limited value.

We suggest defining WM by the following criteria: IgM monoclonal gammopathy; bone marrow infiltration by small lymphocytes, plasmacytoid cells, and plasma cells in a diffuse, interstitial, or nodular pattern; and a surface immunoglobulin–positive, CD19+CD20+CD5–CD10–CD23– immunophenotype.

Waldenström macroglobulinemia (WM) is a chronic B-cell lymphoproliferative disorder characterized by IgM monoclonal gammopathy and bone marrow infiltration by lymphocytes, lymphoplasmacytoid cells, and plasma cells.[1-4] It accounts for approximately 2% of all hematologic malignant neoplasms and has an annual incidence of 6 per 1 million in white males, in whom the incidence is highest.[2,5] The clinical features are highly variable; many patients are asymptomatic, while others have advanced lymphoma. The IgM paraproteinemia may result in hyperviscosity syndrome and cryoglobulinemia, features considered to be highly characteristic of the disorder. The IgM paraprotein also may have autoantibody activity, which may result in peripheral neuropathy, cold agglutinin hemolysis, immune thrombocytopenia, mixed cryoglobulinemia, and acquired von Willebrand disease.[2-4,6,7]

There are no accepted criteria for the diagnosis of WM. The majority of published clinical trials have accepted the presence of IgM monoclonal gammopathy in the context of an apparently indolent lymphoproliferative disorder as sufficient evidence for a definitive diagnosis of WM.[3] IgM paraproteins are, however, demonstrable in all subtypes of peripheral (mature) B-cell lymphoproliferative disorders.[8-10] It has been suggested that paraprotein concentration is useful for differentiating WM from other lymphoproliferative disorders. The French-American-British cooperative group[11] suggested that a concentration of 20 g/L was diagnostic of WM, while Kyle and Garton[9] chose 30 g/L to define WM in a clinicopathologic review of 430 patients with IgM monoclonal gammopathy. In a similar analysis, Owen et al[10] found that paraprotein concentrations do tend to be higher in WM, but there is considerable overlap with other lymphoproliferative disorders and it is not possible to define WM on the basis of IgM paraprotein concentration alone.

The Revised European-American classification of lymphoid neoplasms (REAL)[12] and World Health Organization (WHO) classification criteria[13] consider WM to be a clinical syndrome occurring in most patients with lymphoplasmacytic lymphoma, which is defined as a disorder of "small lymphoid cells that show maturation to plasma cells without the features of other lymphoma types."[12] This category was, however, one of the least reproducible in the "Non-Hodgkin's Lymphoma Classification Project," as expert hematopathologists were able to diagnose it accurately only 56% of the time.[14] The situation is further complicated by the knowledge that lymphoplasmacytic lymphoma may be nonsecretory or indeed associated with IgG or IgA monoclonal proteins.[15-17] The purpose of the present study was to establish whether a combination of clinical, morphologic, and immunophenotypic parameters could be developed to more precisely define WM and permit the identification of prognostic factors. We therefore retrospectively assessed the clinical and laboratory features of 111 cases of WM diagnosed at our institution between March 1993 and May 1999.

Materials and Methods

From March 1993 to May 1999, 111 cases of WM were diagnosed at our regional hematopathology laboratory. A diagnosis of WM was made on the basis of demonstrable IgM monoclonal gammopathy in the context of bone marrow infiltration by lymphocytes, lymphoplasmacytoid cells, and plasma cells. In each case, there were no clinical, morphologic, or immunophenotypic features of other B-cell lymphoproliferative disorders. Particular attention was given to cases with peripheral blood involvement, CD5 expression, and prominent splenomegaly to exclude patients with chronic lymphocytic leukemia (CLL), mantle cell lymphoma, and splenic marginal zone lymphoma. Patients without morphologic or immunophenotypic evidence of bone marrow infiltration were considered to have IgM monoclonal gammopathy of undetermined significance (MGUS) and, therefore, were excluded from this analysis. IgM paraproteins were demonstrated in each case by serum electrophoresis, quantified by densitometry, and typed by immunofixation. The clinical records of 105 cases were made available for review by their primary physicians; survival data were available for all cases.

Assessment of Bone Marrow Morphologic Features

Bone marrow aspirate smears and/or trephine biopsy sections were available for all cases. Morphologic features were reviewed by two of us (R.G.O., A.S.J.), and trephine biopsy infiltration was categorized by consensus as interstitial, diffuse, or focal. *Interstitial infiltration* was defined as generalized marrow involvement but with sparing of normal hematopoiesis and marrow architecture. A *diffuse pattern* was designated if there were confluent areas of infiltration with a loss of hematopoietic elements and fat spaces. *Focal infiltration* indicated foci of infiltration separated by normal residual hematopoietic elements; this was further divided into nodular and paratrabecular patterns. In line with the REAL and WHO criteria,[12,13] no attempt was made to further classify these cases as showing lymphoplasmacytoid, lymphoplasmacytic, or polymorphous histologic patterns. Reticulin staining and the presence or absence of mast cell hyperplasia also were assessed in the majority of cases.

Immunophenotyping

This was performed by 3-color flow cytometric analysis in 98 cases. Briefly, mononuclear cells were isolated from fresh bone marrow aspirates by ammonium chloride lysis, washed in FACSflow/bovine serum albumin (Becton Dickinson Biosciences, San Jose, CA), and resuspended to a final concentration of approximately 20×10^9/L. B-cells were identified by virtue of their side scatter characteristics and CD19 expression. Their immunophenotype was then determined by using the following combinations of monoclonal antibodies (labeled with fluorescein isothiocyanate, phycoerythrin, and Cy5 respectively): CD3/CD3/CD19, CD20/CD5/CD19, FMC7/CD22/CD19, CD11a/CD23/CD19, CD10/CD38/CD19, and kappa/lambda/CD19. Antigens were considered positive when they were detected on at least 50% of the B cells analyzed. Immunophenotype was determined by immunohistochemical analysis (avidin-biotin-peroxidase technique, Dako, Glostrup, Denmark) in the remaining 13 cases. Expression of CD5, CD10, CD20, and CD23 was determined in each case.

Expression of IgD and CD138

Trephine biopsy sections from 69 cases also were assessed for IgD expression within the lymphoid component and CD138 expression within the plasma cell component. Antigen expression was determined by immunohistochemical analysis (avidin-biotin-peroxidase technique) following microwave (and enzymatic for IgD) antigen retrieval.

Statistical Methods

The following parameters (determined at time of diagnosis) were analyzed for their prognostic significance on overall survival: age, paraprotein concentration, pattern of bone marrow infiltration, hemoglobin concentration, pancytopenia, thrombocytopenia, B symptoms, WHO performance status, and serum lactate dehydrogenase (LDH) level. Overall survival was defined as the time from diagnosis to death of any cause, and surviving patients were censored at

the date of their last follow-up. All statistical analyses were performed using SPSS software (SPSS, Chicago, IL). Survival curves for each variable were drawn using the Kaplan-Meier method[18] and compared using the log-rank test.[19] Multivariate analysis was performed using the Cox regression analysis model.[20]

Results

Clinical Features

We included 78 men and 33 women (median age, 71 years; range, 38-91 years) in this retrospective analysis. IgM paraproteinemia was demonstrable in all cases (median concentration, of 16 g/L; range, 2-61 g/L). Immuneparesis and urinary light chain excretion were documented in 18.9% and 47.7% of cases, respectively, while intact paraprotein was detected in the urine of 6.3% of patients. The median plasma viscosity in this cohort was 2.3 mPa (range, 1.6-8.7 mPa).

The case records of 105 patients were made available for review by their primary physicians, and their clinical features are detailed in **Table 1**. Bone marrow infiltration was evident in all cases, while lymphadenopathy, splenomegaly, and overt peripheral blood involvement were documented in 18.1%, 12.3%, and 6.7% of cases, respectively. Many of the "classic" features of WM, such as hyperviscosity syndrome, cryoglobulinemia, and cold agglutinin hemolysis, seemed to be relatively rare in this cohort; they were documented in 5.7%, 3.8%, and 0.0% of cases, respectively. Amyloidosis, large cell transformation, and extranodal disease also were rare, occurring in 1.9%, 4.8%, and 1.0% of cases, respectively. The rarity of amyloidosis and large cell transformation in WM has been documented previously.[21,22] Symptomatic autoimmune phenomena were documented in 14 (13.3%) of 105 cases and consisted of peripheral neuropathy (7 cases), immune thrombocytopenia (4 cases), and acquired von Willebrand disease, myasthenia gravis, and acquired C1 esterase deficiency (Caldwell syndrome; 1 case each). A history of MGUS was obtained in 7.6% of cases.

Table 1
Clinical Features in 105 Cases of Waldenström Macroglobulinemia*

Clinical Feature	No. (Percentage) of Cases
IgM paraprotein (g/dL)	
<10	25 (23.8)
10-19	40 (38.1)
20-29	21 (20.0)
≥30	22 (21.0)
Lymphadenopathy	19 (18.1)
Splenomegaly	13 (12.4)
Asymptomatic disease at diagnosis	35 (33.3)
B symptoms	23 (21.9)
Peripheral blood lymphocytosis (>4,000/μL [>4.0 × 10^9/L])	7 (6.7)
History of MGUS	8 (7.6)
Autoimmune phenomena†	14 (13.3)
Increased lactate dehydrogenase level (>1 times normal) (n = 56)	5 (9.0)
Amyloidosis	2 (1.9)
Cryoglobulinemia	4 (3.8)
Hyperviscosity syndrome	6 (5.7)
Large cell transformation	5 (4.8)
Extranodal disease	1 (1.0)
Chronic renal failure‡	5 (4.8)
Cold agglutinin hemolysis§	0 (0.0)

MGUS, monoclonal gammopathy of undetermined significance.
* It is clear that many of the clinical features thought to be highly characteristic of Waldenström macroglobulinemia, such as hyperviscosity syndrome, cryoglobulinemia, and cold agglutinin hemolysis, seem to be relatively rare.
† The autoimmune phenomena documented were as follows: peripheral neuropathy (7 cases), immune thrombocytopenia (4 cases), acquired von Willebrand disease, myasthenia gravis, and acquired C1 esterase deficiency (1 case each).
‡ Serum creatinine level, >22.6 mg/dL (>200 μmol/L).
§ No cases of clinically significant cold agglutinin hemolysis were documented, but the direct antiglobulin test result was positive in 6 cases.

Bone Marrow Morphologic Features

Bone marrow infiltration was evident in all cases included in this analysis. Morphologic evidence of infiltration was present in 107 (96.4%) of 111 cases. This was manifested by a morphologic excess (>20%) of small lymphocytes on bone marrow aspirate smears and/or evidence of infiltration on trephine biopsy sections. CD20 immunostaining was used to confirm low levels of marrow infiltration in some cases. In the 4 remaining cases, lymphocytes accounted for fewer than 20% of nucleated cells on aspirate smears, but clonal B cells were detected by flow cytometric analysis. Trephine biopsy sections were not available for review in these 4 cases.

Histologic features of trephine biopsy sections were reviewed in 81 (73.0%) of 111 cases, and the cytomorphologic features and pattern of infiltration were determined in each case. In all cases, the sections showed infiltration by small lymphocytes, but the extent of plasma cell differentiation was highly variable. A diffuse pattern of infiltration was documented in 58% (47/81) of cases examined and an interstitial pattern in 32% (26/81). In the remaining cases, the infiltration pattern was nodular (6% [5/81]) or paratrabecular (4% [3/81]). Increased reticulin staining and mast cell hyperplasia were observed in 67% (54/81) and 56% (45/81) of cases, respectively.

Immunophenotype

Immunophenotypic analysis was performed in each of the 111 cases included in this analysis **Table 2**. Three-color flow cytometry was performed in 98 cases and immunohistochemical analysis in the remaining 13 cases. Clonal surface immunoglobulin (sIg) was demonstrated in all cases assessed by flow cytometric analysis, and expression was moderate to strong in virtually all cases. Cell surface light chain

Table 2
Immunophenotypic Profile in 111 Cases of Waldenström Macroglobulinemia*

Antigen	No. (Percentage) of Cases With Positive Antigen Expression
CD19	111 (100)
CD20	111 (100)
CD22	109 (98.2)
CD5	6 (5.4)
CD10	5 (4.5)
CD23	1 (0.9)
IgD (n = 69)	6 (8.7)
CD11a	20 (18.0)
FMC7	77 (69.4)
CD38	42 (37.8)
CD138 (n = 69)	66 (95.7)

* Results are expressed as the number of cases with positive antigen expression. Of the cases, 90.1% (100/111) were characterized by the phenotype CD19+CD20+CD5–CD10–CD23–, while 85.5% (59/69) of cases were CD19+CD20+CD5–CD10–CD23–IgD negative. This is a post–germinal center/marginal zone phenotype.[42]

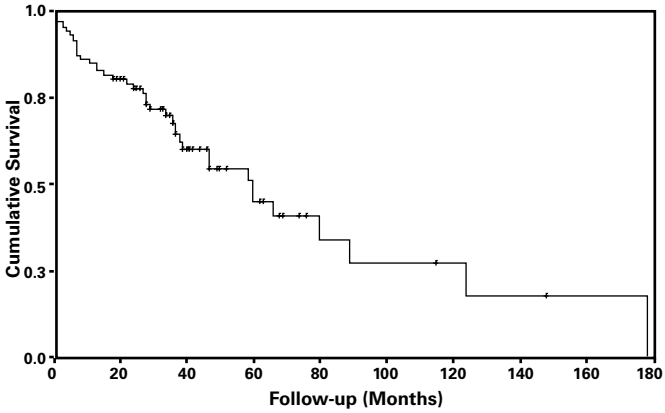

Figure 1 Overall survival in 111 cases of Waldenström macroglobulinemia. The median overall survival from diagnosis in this unselected cohort of patients was 60 months with a median follow-up of 30 months.

expression was identical to the paraprotein light chain in all instances. Demonstration of immunoglobulin light chain restriction on trephine biopsy sections often was not possible owing to the variable degree of plasma cell differentiation seen and the presence of normal plasma cells in some cases (data not shown). CD20 expression was documented in all cases, and antigen expression was moderate to strong in cases assessed by flow cytometric analysis. The majority of cases (90.1%) were characterized by a sIg-positive, CD19+CD20+CD5–CD10–CD23– immunophenotype.

Immunohistochemical analysis also was used to determine IgD expression within the lymphoid component and CD138 expression within the plasma cell component. The majority of cases (91.3%) seemed to be IgD negative, while the plasma cell component was almost invariably (95.7%) CD138+. By using a combination of flow cytometric and immunohistochemical analysis, a CD19+CD20+CD5–CD10–CD23– IgD-negative immunophenotype was demonstrated in 85.5% of cases analyzed. CD11a, CD38, and FMC7 antigen expression was documented in 18.0%, 37.8%, and 69.4% of cases, respectively.

Response to Therapy and Overall Survival

Survival data were available for all cases. The median overall survival from diagnosis was 60 months **Figure 1**, with a median follow-up of 30 months (range, 1-178 months). The minimum follow-up for censored patients was 9 months. Thirty-five (33.3%) of 105 patients were asymptomatic at diagnosis and did not require cytoreductive therapy. Eight of these patients subsequently required treatment. Of the 70 symptomatic patients, 68 received treatment at diagnosis: chlorambucil (n = 53), purine analogues (fludarabine [n = 7], cladribine [n = 1]), or various other treatments (n = 7). The overall response to therapy was 39% (25/64), with no difference documented between patients treated with chlorambucil and those treated with purine analogues. Responses to therapy were deemed to be partial in all cases (defined as a 50% reduction in serum paraprotein concentration and associated mass disease at the completion of therapy).

The median overall survival of patients responding to primary therapy was longer than that of patients with disease resistant to primary therapy (66 vs 47 months), but this difference was not statistically significant ($P = .09$). During the course of follow-up, 48 patients died. Cause of death data were available for 38 of 48 cases. Death was attributed directly to WM in 21 cases (55%) and was considered due to unrelated causes in 17 cases (45)%.

Prognostic Factor Analysis

Univariate prognostic factor analysis **Table 3** showed the following factors to have an adverse effect on overall survival: older than 60 years, WHO performance status more than 1, platelet count less than $100 \times 10^3/\mu L$ ($<100 \times 10^9/L$), pancytopenia, and a diffuse pattern of bone marrow infiltration. The median survival documented in each of these groups was 40, 38, 46, 28, and 59 months, respectively. An increased serum LDH level was of borderline prognostic significance, but it was demonstrable in only 9% (5/56) of cases. In multivariate analysis, age, performance status, and platelet count retained their prognostic significance ($P = .002$, $P = .005$, and $P = .04$, respectively). The IgM paraprotein concentration did not seem to have an effect on overall survival.

Fifty-six patients were stratified according to the International Prognostic Index (IPI)[23] **Table 4**, **Figure 2**. A small number of patients (7% [4/56]) were identified as high risk (IPI 4-5) in whom the outcome was very poor (median

Table 3
Univariate Prognostic Factor Analysis in Waldenström Macroglobulinemia*

Prognostic Factor	*P*
Age, >60 y	.004
World Health Organization performance status, >1	.009
Platelet count, <100 × 10^3/μL (<100 × 10^9/L)	.01
Pancytopenia†	.02
Pattern of bone marrow infiltration (diffuse vs interstitial/focal)	.04
Increased lactate dehydrogenase level (>1 times normal)	.05
B symptoms	.2
Hemoglobin, g/dL (g/L)	
<10 (<100)	.2
<8 (<80)	.35
Paraprotein, g/L	
>10	.36
>20	.65
>30	.4

* Survival curves for each variable were drawn according to the Kaplan-Meier method and compared with the log-rank test using SPSS software (SPSS, Chicago, IL). In multivariate analysis age, performance status, and platelet count retained their prognostic significance (P = .002, P = .005, and P = .04, respectively).
† Pancytopenia was defined as the presence of at least 2 of the following: hemoglobin, <10 g/dL (<100 g/L); WBC count, <4,000/μL (<4.0 × 10^9/L); and platelet count, <100 × 10^3/μL (<100 × 10^9/L).

Table 4
Stratification of 56 Patients According to the International Prognostic Index (IPI)*

IPI Score	No. (%) of Patients
0 (low)	0 (0)
1 (low)	5 (9)
2 (low-intermediate)	34 (61)
3 (high-intermediate)	13 (23)
4 (high)	4 (7)
5 (high)	0 (0)

* The majority (84%) of patients are classified within the intermediate-risk groups. The overall survival of low-risk patients (IPI 0-2) is not statistically different from that of high-risk (IPI 3-5) patients (P = .1; Figure 3).

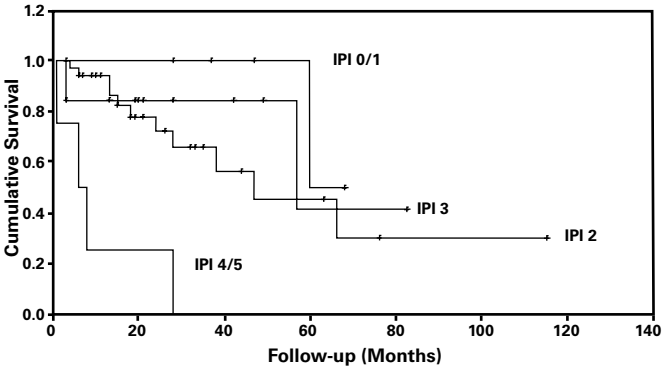

Figure 2 Stratification of patients according to the International Prognostic Index (IPI). In this analysis, the survival of patients classified as IPI high risk was very poor (median survival, <12 mo), but the analysis failed to separate patients classified in the low, low-intermediate, and high-intermediate risk groups.

survival, <12 months), but the majority of patients (84% [47/56]) were classified within the intermediate-risk groups. The overall survival of patients in low-risk (IPI 0-2) groups was not statistically different from that of patients classified within high-risk (IPI 3-5) groups (P = .1) **Figure 3**.

Discussion

WM is a poorly characterized lymphoproliferative disorder. There have been very few detailed clinicopathologic assessments, while the majority of clinical trials have been nonrandomized, single-institution, phase 2 studies. Progress in this area has been hindered by the lack of accepted diagnostic criteria. The majority of published studies to date have accepted the presence of IgM monoclonal gammopathy in the context of an apparently indolent lymphoproliferative disorder as sufficient evidence for the diagnosis of WM.[3] This is unsatisfactory, and criteria incorporating clinical, morphologic, immunophenotypic, and, ultimately, genotypic parameters are needed for the accurate diagnosis of WM.

IgM paraproteinemia cannot be regarded as diagnostic of WM, as it has been shown that this is demonstrable in all subtypes of peripheral (mature) B-cell disorders.[8-10] The French-American-British group and others have suggested that paraprotein concentration is useful for differentiating WM from other lymphoproliferative disorders.[9,11] However, in a recent analysis of 106 cases of IgM monoclonal gammopathy, Owen et al[10] were unable to identify a concentration that clearly differentiated WM from other B-cell disorders. It also is apparent from this analysis that many of the classic clinical features, such as hyperviscosity syndrome, cryoglobulinemia, and cold agglutinin hemolysis, seem to be relatively rare and, therefore, should not be used as defining features.

It is clear that immunophenotypic criteria are needed to differentiate WM from other indolent lymphoproliferative disorders. San Miguel and colleagues[24] used indirect immunofluorescence in 12 cases and demonstrated the phenotype CD9+CD20+HLADr+FMC7+, while CD10 expression was seen in 2 of 4 cases and CD5 expression in 1 of 3 cases. The expression of pan-B-cell antigens CD19, CD20, and CD22 has been confirmed by flow cytometric and immunohistochemical analysis and alkaline phosphatase-anti–alkaline phosphatase (APAAP) techniques.[25-27] Hall et al[26] examined 18 cases of lymphoplasmacytoid lymphoma by immunohistochemical analysis and demonstrated a CD19+CD20+CD22+CD10– phenotype, while CD5 positivity was demonstrated in 40% of cases. However, only one third of the cases had demonstrable IgM paraproteins, while CD5 positivity was associated significantly with

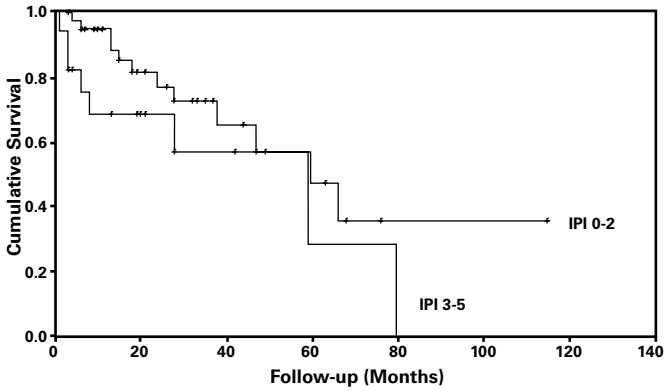

Figure 3 The overall survival of patients stratified as low risk (International Prognostic Index [IPI] 0-2) is not statistically different from that of patients classified as high risk (IPI 3-5; $P = .1$).

peripheral blood lymphocytosis, suggesting an alternative diagnosis of CLL in at least some of these cases.[26] Similarly, Schwonzen and colleagues[27] demonstrated (by APAAP studies) a CD5+CD10–CD19+CD23+CD38+ immunophenotype in 15 cases of lymphoplasmacytic lymphoma, but only 4 of these cases had an IgM paraprotein, while 13 had a leukemic presentation.

In the present study, we used 3-color flow cytometric and immunohistochemical analyses to examine the immunophenotype of 111 cases of WM diagnosed according to REAL/WHO criteria. We found that these cases were consistently characterized by an sIg+CD19+CD20+CD5–CD10–CD23– immunophenotype. The majority of cases were also negative for IgD by immunohistochemical analysis, while the plasma cell component was almost invariably CD138+. In more recent cases evaluated by 3-color flow cytometric analysis, we have shown that surface IgM is always present, but a significant proportion of cases also express IgD to some extent (data not shown). Our results are in keeping with those of Matutes et al,[28] who demonstrated a CD5–CD23– immunophenotype in the majority of 25 cases of lymphoplasmacytic lymphoma examined by single-color flow cytometry.

The CD19+CD20+CD5–CD10–CD23– immunophenotype differentiates WM from the majority of CLL and mantle cell lymphoma cases, but not necessarily from follicular lymphoma (FL), as the expression of CD10 and CD23 in the latter seems to vary considerably.[12,13] Indeed, CD10 expression in FL often is confined to the cells contained within the follicles of lymph nodes and is not demonstrable in extrafollicular neoplastic cells.[29] A paratrabecular pattern of bone marrow infiltration is characteristic of FL, while nodular and interstitial patterns seem to be rare. Paratrabecular infiltration was demonstrated in only 3.7% of WM cases assessed in the present study. Although it may be possible to differentiate WM from FL by cytomorphologic features, it would seem prudent to advise lymph node biopsy and/or t(14;18) polymerase chain reaction in cases with a purely paratrabecular pattern of infiltration.

CD20 antigen expression was documented in each of the 111 cases examined in the present study, and antigen density was moderate to strong in cases assessed by flow cytometric analysis. In this context, it is interesting to note that rituximab therapy has been used with some success in patients with relapsed or refractory WM.[30-32]

WM is perceived by many to be an indolent disorder. However, the median overall survival from diagnosis in this unselected cohort of patients was only 60 months, which is significantly shorter than that documented for patients with CLL and FL. A similarly short median survival was documented in previous WM studies[2,21,33,34] and by the Non-Hodgkin's Lymphoma Classification Project for patients with lymphoplasmacytic lymphoma.[35] The overall response to initial therapy in the present analysis was only 39.1%, with no apparent difference seen between patients treated with chlorambucil and those treated with purine analogues. The overall survival of patients responding to initial therapy in the present study was longer than that of patients with disease resistant to primary therapy (66 vs 47 months). This trend also has been documented in previous studies.[2,3,21] The most appropriate primary therapy has not, however, been established for WM, and a trial of chlorambucil vs fludarabine or cladribine would seem appropriate. In the present analysis, it was estimated that in 55% of cases, death was directly attributable to WM but was due to unrelated causes in the remaining patients. Interpretation of these data is not, however, straightforward, as it has been shown that fairly modest increases in plasma viscosity are associated with an increased risk of death due to ischemic heart disease and stroke.[36-40]

Univariate prognostic factor analysis demonstrated that age, performance status, platelet count, pancytopenia, and a diffuse pattern of bone marrow infiltration were unfavorable factors. In multivariate analysis, age older than 60 years, performance status more than 1, and platelet count less than $100 \times 10^3/\mu L$ ($<100 \times 10^9/L$) retained their prognostic significance, with P values of .002, .005, and .04, respectively. Facon et al,[21] in an analysis of 167 patients, demonstrated that male sex, a neutrophil count less than 1,700/μL ($<1.7 \times 10^9/L$), age older than 60 years, and a hemoglobin concentration of less than 10 g/dL (100 g/L) were independent adverse prognostic indicators.

The IPI did not successfully stratify patients in this cohort, as 84% were classified within the intermediate risk groups (IPI 2 and 3). This may reflect the small number of cases included in the analysis, but it is noteworthy that the Non-Hodgkin's Lymphoma Classification Project was similarly unable to demonstrate its significance in a small group

of lymphoplasmacytic lymphomas.[35] It is not, however, surprising that the IPI seems to be less applicable to WM; the majority of patients are older than 60 years and by definition have stage IV disease, while an increased LDH level and extranodal involvement are rare (8.9% and 1.0%, respectively, in this cohort). A binary classification system has been advocated by Gobbi and colleagues,[33] in which 4 independent prognostic variables (age, hemoglobin concentration, weight loss, and cryoglobulinemia) are used to define 2 prognostic groups. Possession of one or fewer of these factors identifies a "good-risk" group, while "poor-risk" patients have 2 or more of these adverse prognostic factors.[33] Similarly, Morel and colleagues[34] proposed a prognostic scoring system based on age, serum albumin level, and the number of peripheral blood cytopenias. In this model, patients are stratified into low-, intermediate-, and high-risk groups that are associated with 5-year survival rates of 87%, 62%, and 25%, respectively.[34] Hemoglobin concentration was not a significant variable in the present study. This is difficult to explain, but Dimopoulos and Alexanian[2] were similarly unable to demonstrate any prognostic significance in their analysis. Thrombocytopenia and pancytopenia were, however, significant factors, and it may be that these parameters are more sensitive indicators of bone marrow infiltration than hemoglobin concentration.

Paraprotein concentration did not seem to have prognostic significance in the present analysis. This was a little surprising, but it has been documented in a number of studies.[2,21,33] In addition, the overall survival in lymphoplasmacytic lymphoma cases classified as WM on the basis of IgM monoclonal gammopathy is not statistically different from that of non–IgM-producing lymphoplasmacytic lymphomas[16] (data not shown). The IgM paraprotein levels are a continuous variable in WM that do not seem to correlate with the extent of bone marrow infiltration.[41] The latter does, of course, have prognostic significance; in the present study, a diffuse pattern of infiltration was associated with an inferior outcome. This is consistent with the results of Bartl et al,[41] who estimated the extent of marrow infiltration by histomorphometry and found that the extent of marrow disease correlated with survival. In the latter study, patients also were described as having lymphoplasmacytoid (predominantly small lymphocytes), lymphoplasmacytic (mixture of small lymphocytes and plasma cells), or polymorphous (mixed cell population consisting of small lymphocytes, plasma cells, and blast cells) histologic patterns. Outcome was good in the lymphoplasmacytoid group, intermediate in the lymphoplasmacytic group, and poor for those with a polymorphous pattern. These differences are likely to reflect differences in the extent of marrow infiltration rather than true cellular differences, as the percentages of patients with more than 50% infiltration were 7%, 34%, and 84% in these groups, respectively.[41]

In the present study, we have shown that WM is characterized by a CD19+CD20+CD5–CD10–CD23– immunophenotype. This is a post–germinal center or marginal zone phenotype[42] and suggests that the disorder may arise from marginal zone memory B cells. This hypothesis also is supported by immunoglobulin variable region sequence analysis, as extensive somatic hypermutation is demonstrable in most cases without intraclonal variation.[43-46] It may be appropriate to consider WM to be a marginal zone lymphoma forming a continuous spectrum with splenic and nodal marginal zone lymphomas. Indeed, Owen et al[47] were unable to demonstrate significant phenotypic or survival differences between cases diagnosed with WM and those diagnosed with splenic marginal zone lymphoma. It also is interesting to note that in their recent analysis of 124 cases of non–mucosa-associated lymphoid tissue marginal zone lymphoma, the Lyon group found that a significant proportion were not classifiable as "nodal" or "splenic," and that a significant minority of patients had IgM paraproteinemia.[48]

There also are some limited cytogenetic data to support this "unifying" hypothesis. Additional copies of chromosomes 3 and 18 have been reported by a number of investigators in WM[47,49-53]; these are, of course, the most common numeric abnormalities identified in marginal zone lymphoma.[42] Likewise, the t(11;18) has been described in 2 patients with WM[54,55]; this translocation occurs in approximately 40% of extranodal marginal zone lymphomas and results in a novel *API2-MLT* fusion gene.[56] A t(9;14) has been described in lymphoplasmacytic lymphoma and a variant t(2;9;14) in a case of splenic marginal zone lymphoma.[57-59] There is, therefore, accumulating evidence in favor of classifying WM as a marginal zone lymphoma. It may, therefore, be appropriate to consider WM, non-IgM lymphoplasmacytic lymphoma and splenic and nodal marginal zone lymphomas as part of the same spectrum of disease, which may be termed *systemic marginal zone lymphoma*.

WM is a poorly characterized lymphoproliferative disorder. It has an inferior outcome compared with other indolent lymphomas and is associated with poor response rates to conventional chemotherapeutic agents. Clinical trials evaluating conventional and novel therapies are needed, but these will require stringent diagnostic criteria to accurately define WM. We suggest that WM be defined by following criteria: IgM monoclonal gammopathy of any concentration; bone marrow infiltration by small lymphocytes, plasmacytoid cells, and plasma cells in a diffuse, interstitial, or nodular pattern; and possession of an sIg+CD19+CD20+CD5–CD10–CD23– immunophenotype **Table 5**.

Table 5
Proposed Criteria for the Diagnosis of Waldenström Macroglobulinemia

- IgM monoclonal gammopathy of any concentration
- Bone marrow infiltration with small lymphocytes, plasmacytoid cells, and plasma cells
- Diffuse, interstitial, or nodular pattern of bone marrow infiltration*
- Immunophenotype: sIg+CD19+CD20+CD5–CD10–CD23–

sIg, surface immunoglobulin.
* A paratrabecular pattern of bone marrow infiltration does not preclude a diagnosis of Waldenström macroglobulinemia but is more suggestive of follicular lymphoma. Lymph node biopsy and/or t(14;18) polymerase chain reaction analysis is advisable in these cases.

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